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THE EFFECT OF LOW INTENSITY PULSED ULTRASOUND TREATMENT COMBINED WITH MESENCHYMAL STROMAL CELL INJECTION FOR CARTILAGE REGENERATION IN A KNEE OSTEOCHONDRAL DEFECT MODEL OF RATS.

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Purpose: Once cartilage is injured, it rarely recovers spontaneously, because of their poor intrinsic healing capacity. Cell transplantation therapy is anticipated to regenerate cartilage defect. Mesenchymal stromal cell (MSC) is one of expecting cell sources for cartilage repair due to their character including the capability which differentiate into chondrocyte. However there were few study that verified efficacy and safety of aftertreatment post cell transplantation. There were some reports that low intensity pulsed ultrasound (LIPUS), which is used for bone fracture treatment, could stimulate MSC differentiation into osteo-/chondro-cyte in vitro. The aim of this study was to investigate whether LIPUS treatment combined with cell therapy could affect cartilage regeneration for a knee osteochondral defect model of rats.

Methods: This study was approved by the animal research committee of our facility. An osteochondral defect of 1mm diameter was created on both femur grooves of twelve Wistar rats at 12-week old. Four weeks after creation of the defect, 1.0×10^6 allogeneic bone marrow MSCs diluted with phosphate-buffered saline (PBS) was transplanted into right knee joint by intra-articular injection and PBS without MSC was injected into left knee joint. The rats were divided into 2 interventions: without or with LIPUS treatment. Two days after injection, the rats with LIPUS were subjected to LIPUS treatment according to parameters borrowed from those for bone fracture treatment, 20 min/day, 5 days/week, to both knee joints. After 4 and 8 weeks intervention, the rats were euthanized, femora were removed and divided into four groups: Control group (PBS injection), LIPUS group (PBS injection with LIPUS treatment), MSC group (MSC injection) and MSCL group (MSC injection with LIPUS treatment). The 6- μ m thick serial sections of the femur specimen stained with safranin-O and hematoxylin-eosin were examined and scored with Wakitani's cartilage repair score. The collagen type I and II expressions were also observed by immunohistochemical methods.

Results: Four weeks after intra-articular injection, the histological score were as follows, Control group: 8.7 ± 2.36 , LIPUS group: 4.7 ± 1.31 , MSC group: 4.7 ± 1.31 , MSCL group: 4.3 ± 0.65 . The defect area was filled with repair tissue which wasn't hyaline cartilage in Control group. Repair tissue in Control group was mostly expressed by collagen type I, but collagen type II expression was restricted in deep zone. In LIPUS, MSC and MSCL group, repair tissue mostly included hyaline cartilage like cell morphology, and showed SO staining intensity in middle zone. The repair tissue in these three group was thicker than Control group. The expression of collagen type II was observed in wide range of repair tissue in LIPUS, MSC and MSCL group, but the expression of type I collagen was observed through surface and middle zone in these groups. Eight weeks after intra-articular injection, the histological score were as follows, Control group: 7.7 ± 2.36 , LIPUS group: 7.0 ± 1.96 , MSC group: 4.7 ± 1.31 , MSCL group: 4.0 ± 0.00 . In Control and LIPUS group, fibroblast like cell morphology was observed and SO intensity was reduced. The expression of collagen type II was attenuated through surface to middle zone in Control group, while attenuated in surface zone of LIPUS group. In MSC and MSCL group, hyaline cartilage like cell morphology was observed in repair tissue, but the SO intensity was reduced. The expression of collagen type II was attenuated in surface zone of MSC and MSCL group. The expression of collagen type I was located in surface zone of all group or middle zone in some specimen.

Conclusions: It might be indicated LIPUS treatment or MSC injection could stimulate cartilage regeneration in 4 weeks after MSC injection, but repaired cartilage stimulated by LIPUS treatment was deteriorated in 8 weeks after MSC injection. In this experiment condition there might be little interactive effect between LIPUS and MSC injection for cartilage repair.

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THE EFFECT OF SYSTEMIC ADMINISTRATION OF GRANULOCYTE-COLONY STIMULATING FACTOR (G-CSF) ON FULL THICKNESS CARTILAGE DEFECT IN A RABBIT

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Purpose: Bone marrow stimulation is in use clinically as a treatment option for cartilage defects. Theoretically mesenchymal stem cells reside in bone marrow are induced into cartilage defects followed by cartilage repair with this technique. Although mostly good clinical results were reported, histology revealed repaired cartilage was fibro-to hyaline-like cartilage.

The aim of this study is to investigate whether systemic administration of G-CSF, stimulant of bone marrow, could improve the quality of repaired tissue using full thickness articular cartilage defect model of a rabbit.

Methods: Thirty 12week-old male New Zealand White rabbits were divided into three groups.

The low dose group (n=10) received daily 10 μ g/kg of G-CSF, the high dose group (n=10) 50 μ g/kg, subcutaneous injections for three days, prior to creating cartilage defects. To the control group (n=10), saline was administered for three days.

48 hours after the first injection, a 5.2mm diameter cylindrical osteochondral defect was created in the center of the femoral trochlea.

4, 12 weeks after the procedure, status of repaired tissue was evaluated by macroscopically as well as microscopically.

Results: Macroscopically, the defect fillings and the tissue qualities were better in G-CSF group than in the control group at 4 weeks. (High dose group showed better than the low dose group.)

Qualities of repaired cartilage were better in the G-CSF group at 12 weeks but the defect fillings were better in the control group, which assumed to be hypertrophy of fibrocartilage.

Conclusions: Macroscopically, G-CSF administration prior to bone marrow stimulation was effective in modifying quality of repaired tissue.

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INTRODUCTION OF MESSENGER RNA INTO KNEE ARTICULAR CARTILAGES USING POLYPLEX NANOMICELLE

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Purpose: Osteoarthritis (OA) is a degenerative joint disease that is caused by an imbalance in cartilage degeneration and synthesis, which results in pain and low quality of life in patients. It is a major health problem in elderly generation. Besides painkillers and anti-inflammatory drugs alleviating OA symptoms, disease-modifying osteoarthritis drugs (DMOADs) have drawn much attention. Given the fact that gene products stimulate chondrogenesis or break down cartilage matrix, delivery of therapeutic genes as DMOADs to the articular cartilage is a promising strategy for the treatment of OA. However, there are concerns regarding safety and efficiency on the introduction of nucleic acids in vivo. Although viral transduction shows high efficiency of gene introduction, its application is limited because of strong immunogenicity and toxicity. Plasmid DNA transfection has a risk of insertion into host genome. Messenger RNA (mRNA) introduction would directly induce the expression of therapeutic proteins in target cells without any risk of insertion mutagenesis. A biocompatible gene carrier based on self-assembly of a polyethylene glycol (PEG)-polyamino acid block copolymer, polyplex nanomicelle, was recently shown to achieve in vivo mRNA introduction by solving two major limitations for the in vivo mRNA delivery, instability and immunogenicity of mRNA. In this study, we examined the efficacy of the polyplex nanomicelle-mediated mRNA introduction into the articular cartilage of intact or surgically-induced OA knees in mice, aiming to apply this strategy to the treatment of OA